RHEOLOGY OF PREBIOTIC CHOCOLATE BAR

THESIS

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By

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I. Introduction

I.1 Galacto Oligosaccharides and Health Benefits

Oligosaccharides have been known as a functional food ingredients that have a great potential to improve the quality of foods. Current major uses of oligossacharides are in beverages, infant milk powders, confectionary, and dairy desserts. Oligosaccharides are water-soluble and have a mild sweetness, which are suitable for food that needs a bulking agent with reduced sweetness.

Commercially available Galacto Oligosaccharide (GOS) products are mixture of galactose based oligossacharides of varying degree of polymerization (DP) and linkage configuration with glucose, galactose and lactose (Charampopoulos & Rastall, 2009). In the United States, GOS has been accepted to be Generally Recognized as Safe (GRAS). Toxicological studies of GOS have shown that GOS has no significant effect on clinical pathologies (Anthony et al., 2006). GOS is not digestibile and thus is similar to cellulose and other fibers (Charampopoulos & Rastall, 2009).

GOS is a prebiotic. The establishment of bifidus microflora, which are commonly used to produce yogurt, in the intestines of breast-fed infants has been attributed to the presence of galacto containing oligosaccharides in human milk (Smart, 1993). Prebiotics are non-digestable ingredient that beneficially affects the host by selectively stimulating the growth and activity of one or a limited number of bacterial species already resident in the colon (Gibson & Roberfroid, 1995).

Health benefits attributed to GOS as reduction of the level of cholesterol in serum, colon cancer prevention, and enhancement of mineral absorption (Lim and others 2005; Pool-Zobel and Sauer 2007; Liong 2008; van den Heuvel and others 2000; Chonan and others 2001; Alliet and others 2007)In addition, there are 2 notable studies that involve GOS digestion showing its chemopreventive effect in rat models. Benign and malignant tumors in DMH (dimethylhydrazine) treated rats have been significantly reduced in a diet of 27% GOS in low, medium, and high fat diets (Wijnands and others 1999). Additionally, GOS has been shown to have anticarcinogenic effect when comprising 20% of the diet using AOM (azoxymethane) treated rats. It was found that aberrant crypt foci (ACF, a precursor for colon cancer, eventually forming colorectal polyps) multiplicity was significantly lowered. However, this was the only indication of GOS having a protective effect against colon tumors (Wijnands and others 2001). These studies show GOS effectiveness against colon cancer in animal models. However, studies in humans have not been conducted yet.

I.2 Rheology

Rheology is defined as the science of deformation and flow of matter and it is considered as a critical parameter for assessing chocolate quality. Three main parameters used in the rheological assessment of chocolate are yield stress, plastic viscosity, and thixotropy. Yield stress is the amount of energy that is required to start flow. Plastic viscosity is the amount of energy required to keep the chocolate flowing. Yield stress is critical in relation to shape retention, pattern holding, inclined surface coating, and air bubbles. The plastic viscosity relates to the ease of pumping, filling, and coating properties. Thixotropy shows the amount of conching process of the chocolate.

Chocolates that are properly conched would be thixotropic. Rheology is important for quality control/assurance and able to show differences in composition, processing, and particle size distributions (Afoakwa and others 2008). Flavor perception and sensory qualities are related to rheology of chocolate (Ziegler and others 2001).

Previously, the Casson equation was recommended for the rheological parameters with a heated rheometer with bob and cup geometry. The Casson equation has been shown to have significant inconsistencies between different laboratories measuring the same chocolate (Aeschlimann and Beckett 2000). Therefore, a new method was proposed and published (ICA 2000). The new method had a pre-shear at 5 s^{-1} for greater than 5 minutes and measurement of stress and viscosity was obtained using a shear rate ramp up and down from 2 s^{-1} to 50 s^{-1} and 50 s^{-1} to 2 s^{1} (ICA 2000). Further research has shown a more accurate method that could be verified for different systems (Servais and others 2003). ICA method had been shown to have a higher correlation and regression coefficients when compared to the Casson equation method (Afoakwa and others 2009). The two methods had been shown to have a high correlation; therefore, either method could be used to analyze the rheological parameters of chocolate (Afoakwa and others 2009).

II. Material and Methods

II.1. Chocolate

The chocolate was formulated for Control, 3.75% GOS, 15.4% GOS, and 30.8% GOS were a typical dark chocolate formulation. GOS was used to replace sucrose in the chocolate formulation. The formulations for the chocolate samples are shown in Table 1. The ingredients were provided from the following sources: chocolate (Chocolate Liquor, LQ-6251, ADM Cocoa; Milwaukee, Wisconsin), sucrose (Purchased at Kroger, Sugar, Premium Pure Cane Granulated, Net Weight 1.81 kg, Manufacture Code 4014 4A, Domino Foods; Yonkers, New York), GOS Purimune™ (Galactooligosaccharides, GO-P 90, 90.5% GOS Dry Basis, Product ID 113001-156, Lot 15271, Manufactured 12/16/2008 in Korea, GTC Nutrition; Golden, Colorado), cocoa butter (Prime Cocoa Butter, ADM Cocoa; Milwaukee, Wisconsin), and soy lecithin (Ultralec® G, Deoiled Lecithin, Product Code 700853, Lot Number 08122U062, ADM; Decatur, Illinois). The calculated amount of fat in each sample was 35.4% and is considered a 61.1% chocolate bar. Oil (Vegetable Oil, Kroger Company; Cincinnati, Ohio) was used in the conching oil bath.

Table 1. Sample formulations by weight percent.

Ingredient	Control	3.75% GOS	15.4% GOS	30.8% GOS
Chocolate	51.90%	51.9%	51.96%	51.96%
Sugar	38.42%	34.68%	22.99%	7.6%
GOS Purimune	0.0%	3.8%	15.39%	30.8%
Cocoa Butter	9.2%	9.2%	9.16%	9.2%
Soy Lecithin	0.5%	0.5%	0.50%	0.5%
Total Mass	100.0%	100.0%	100.0%	100.00%

II.3 Chocolate Processing

The chocolates samples were produced in a manner that maintained identical processing for all of the chocolate samples. The first part of the processing, refining, was performed in the dry pilot plant in the Food Science Building at Pennsylvania State University (University Park, Pennsylvania). Temperature of the pilot plant was at 21.7 °C with 31% Relative Humidity. The chocolate, sucrose, and GOS Purimune™ were weighed and combined for all four samples. The mixtures were heated at 50 °C using a proofing cabinet with no water (Proofbox GS, MIWE; Hillsborough, New Jersey) until the chocolate was melted. The melted chocolate and powder mixture was stirred until all of the powder was incorporated in the chocolate. The Control sample was refined (Figure 1) first to minimize cross-contamination of GOS using a 3-roll, lab scale apparatus cooled with circulating tap water (Lehmann Model 5B, Mullins Manufacturing Corp.; Salem, Ohio). The chocolate samples went through the refiner twice. The rollers were cleaned and reset in between runs and samples. After each sample was refined twice, a micrometer (02-223-005, Fowler; Newton, Massachusetts) was used to determine the particle size. The chocolate flake was placed in mineral oil and then placed onto the micrometer for measurement. All chocolate samples had a particle size of 25 µm and were packaged in plastic containers before transporting back to Columbus, Ohio for further processing.

Figure 1. Chocolate refining.

Conching is an important step in chocolate production. The process was completed using a mixer (KitchenAid®, Model KSMC50S; St. Joseph, Michigan) with a paddle attachment. The mixer bowl was surrounded with a jacket (KitchenAid®, Model K5AWJ; St. Joseph, Michigan) to allow uniform heat treatment with an oil bath. A hot plate was used to heat the oil and circulated into the jacket using a small pump (Little Giant©, Model 1-AA-OM; Oklahoma City, Oklahoma). Figure 2 shows a photograph of the conching system. The conching process was performed in a small room with a dehumidifier to help maintain a low humidity environment. Low humidity environment is needed in order to decrease moisture migration into the chocolate during the chocolate production. The four samples were processed for 5.5 hr on separate days. The chocolate flake for each sample was conched for 5 hours with "stir" speed. Cocoa butter and soy lecithin were added for the last 0.5 hours. The temperature of the chocolate during the conching process was maintained near 48 °C for 6 hours without the come up time.

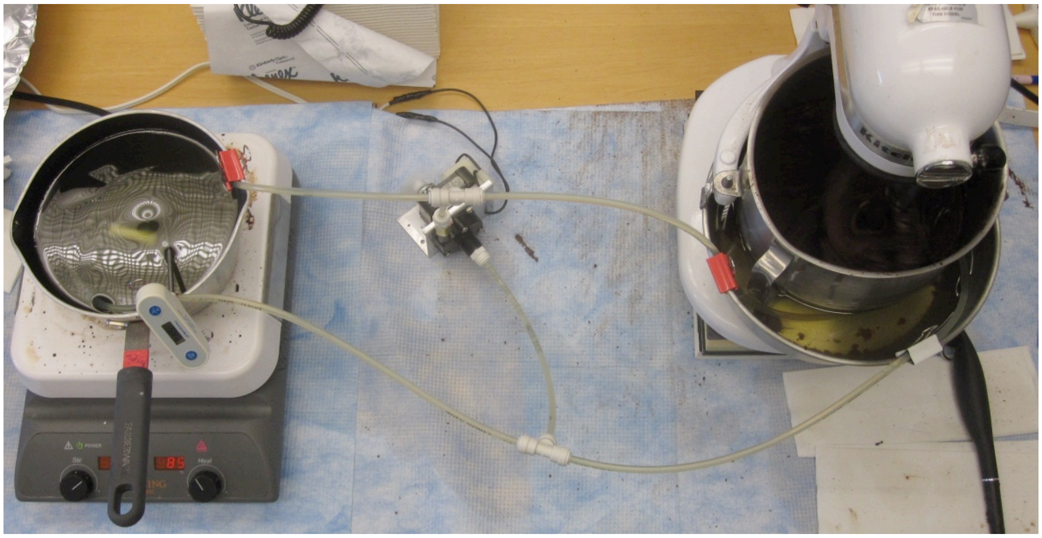

Figure 2. Conching process.

The tempering process was done using a lab size unit tempering machine (Revolation 2, ChocoVision Corporation; Poughkeepsie, New York) as shown in Figure 3. The conched chocolate samples were placed into the tempering machine as needed. Preset tempering cycle was chosen initially and Chocolate Type – Dark Chocolate and Temper 1 Cycle were then used as the setting for the tempering. Prior to starting the tempering cycle, the chocolate was melted completely and was maintained at 42 °C for 30 minutes. When the tempering cycle began, the temperature was brought down to 31.5 °C until the tempering was completed. Once the tempering process was done, approximately 100 grams of tempered chocolate was poured into plastic molds. Then, the chocolate was cooled on racks in a 20 °C room overnight to let the samples hardened.

Figure 3. Chocolate tempering.

II. 3 Rheological Measurements

.The chocolate samples were analyzed in triplicate to compare yield stress, apparent viscosity, and thixotropy between the four samples (Afoakwa and others 2009). 20 g of chocolate was weighed in a beaker and placed in an oven set above 50 °C (Oven range recorded 52 °C to 56 °C). The chocolate was melted for more than 75 minutes (Samples removed after 80- 90 min of melting) in order to remove fat crystals from the sample. A rheometer (AR 2000 ex, TA Instruments; New Castle, Delaware) was calibrated and heated to the sampling temperature of 40 °C. The calibration included inertia, geometry inertia, bearing friction, zero gap, and rotational mapping (type: precise). Attached to the rheometer was a concentric cylinder (AR 2000 ex accessory, TA Instruments; New Castle, Delaware) and DIN geometry (DIN Rotor, Part 545012.901, TA Instruments; New Castle, Delaware) with a ratio of inner to outer radius 0.93. A photograph of the filled concentric cylinder and rheometer with cylinder attachment is shown in Figure 4. To maintain temperature and prevent crystal formation, the chocolate samples from the oven were transferred immediately into the cup geometry (18.11 ± 0.048 g). The geometry was lowered to the testing gap (5920 μm) and a solvent trap was placed over the cup opening to help insulate the concentric cylinder. The rheometer experimental procedure involved the following steps all at 40°C. The samples were pre-sheared for 15 minutes at 5 s^{-1}. The rheometer began to ramp up the shear rate from 5 s^{-1} to 50 s^{-1} in a 2 min interval followed by ramping down the shear rate from 50 s^{-1} to 5 s^{-1} in a 2 min interval. Data points were recorded every second to allow measurements to be recorded closest to the key shear rates for yield stress (stress value during ramp up at 5 s^{-1}), apparent viscosity (viscosity value during ramp up at 30 s^{-1}), and thixotropy (difference of stress at shear rate of 5 s^{-1} between ramp up and down). The data was collected by Rheology Advantage Instrument Control AR (Version 5.7.0, TA Instruments; New Castle, Delaware) and analyzed using Rheology Advantage Data Analysis (Version 5.6.0, TA Instruments; New Castle, Delaware). The averaged values were calculated for each attribute and analyzed for statistical differences (ANOVA, $\alpha = 0.05$). The statistical analysis was performed using Minitab 16 (Minitab Inc.; State College, Pennslyvania).

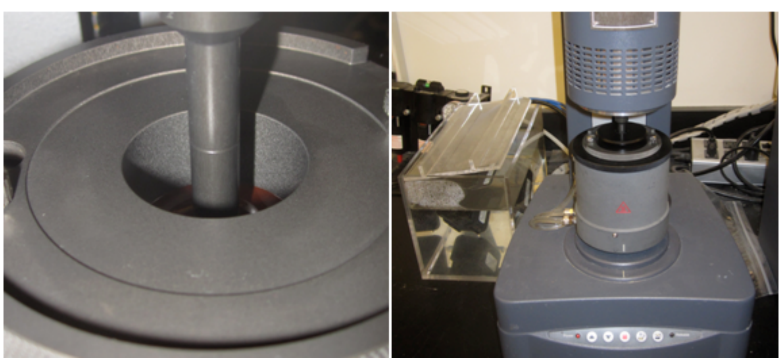

Figure 4. The chocolate filled cylinder (Left) and the rheometer fitted with concentric cylinder during measurement (Right).

III. Result and Discussion

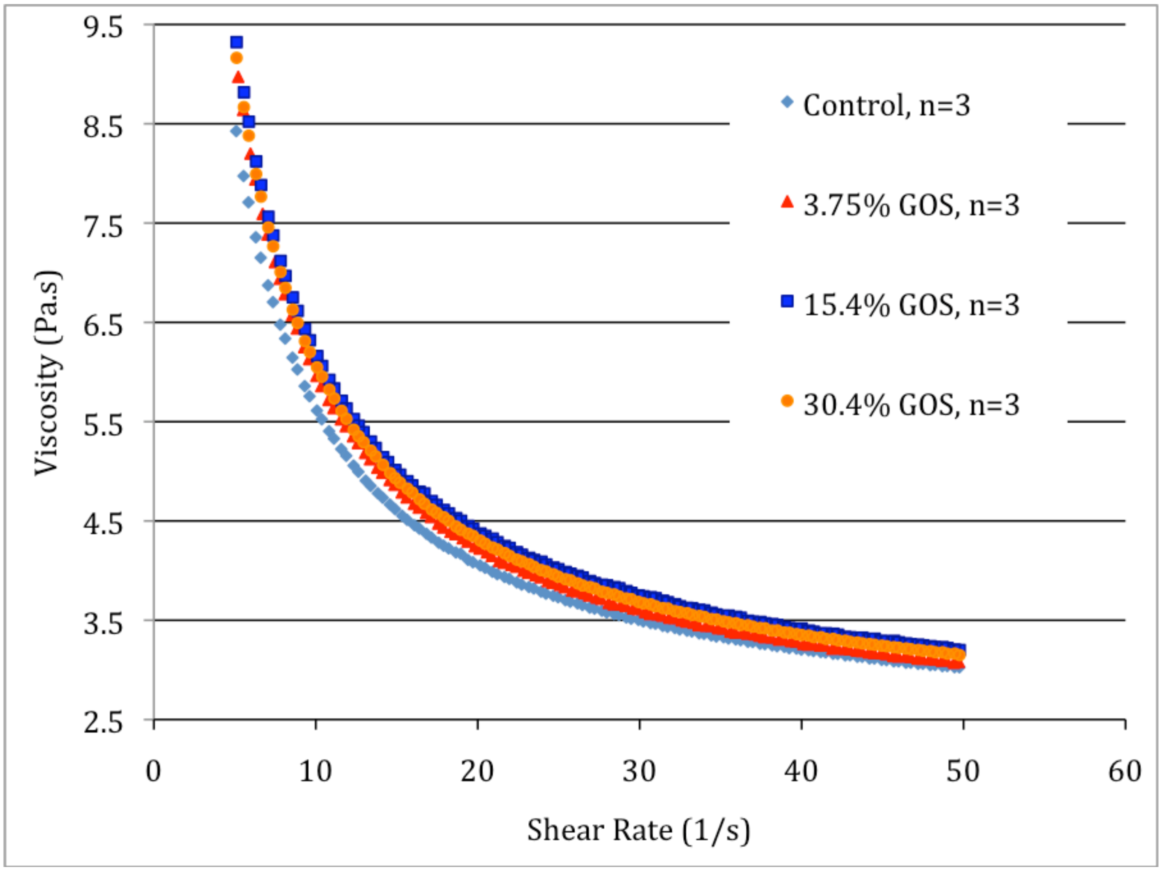

Figure 5. Viscosity measurements during shear ramp-up for all chocolate samples (Apparent viscosity at 30 s^{-1}).

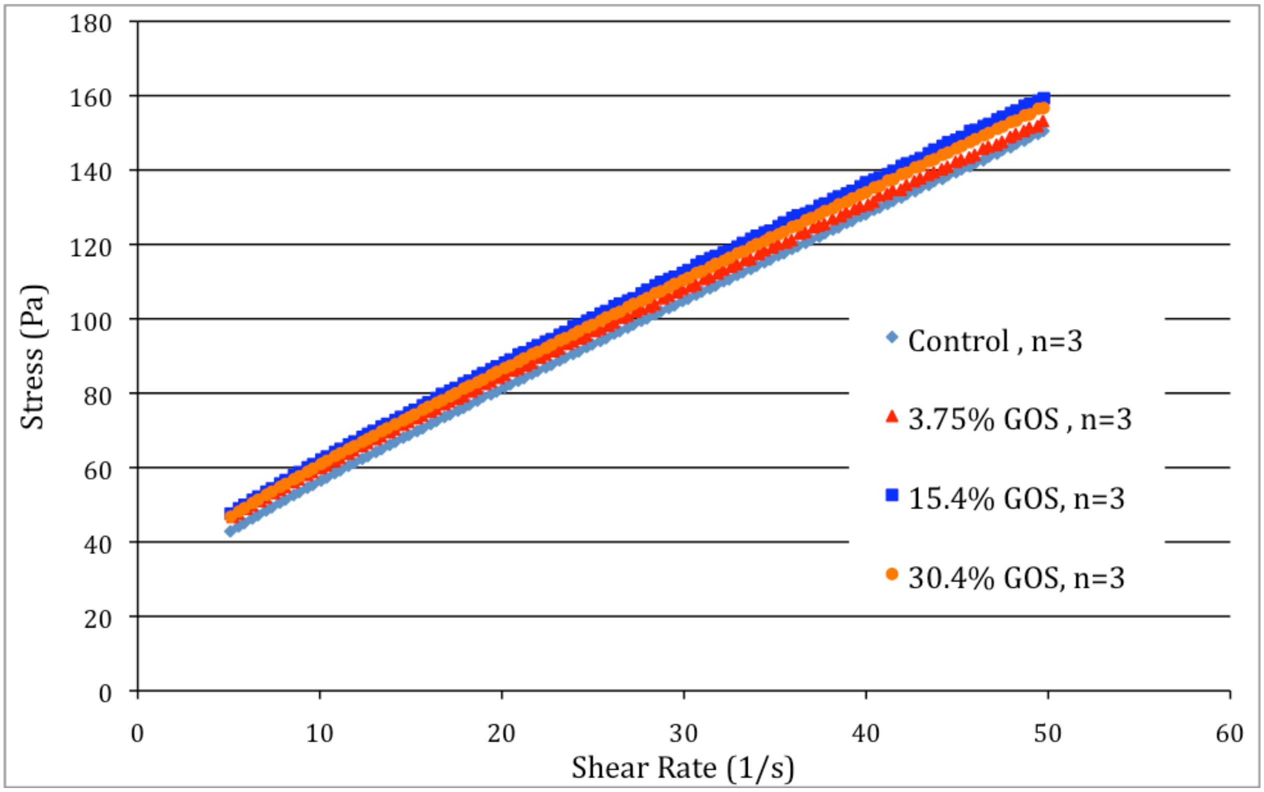

Figure 6. Stress measurements during shear ramp-up for all chocolate samples (Yield Stress at 5 s^{-1}).

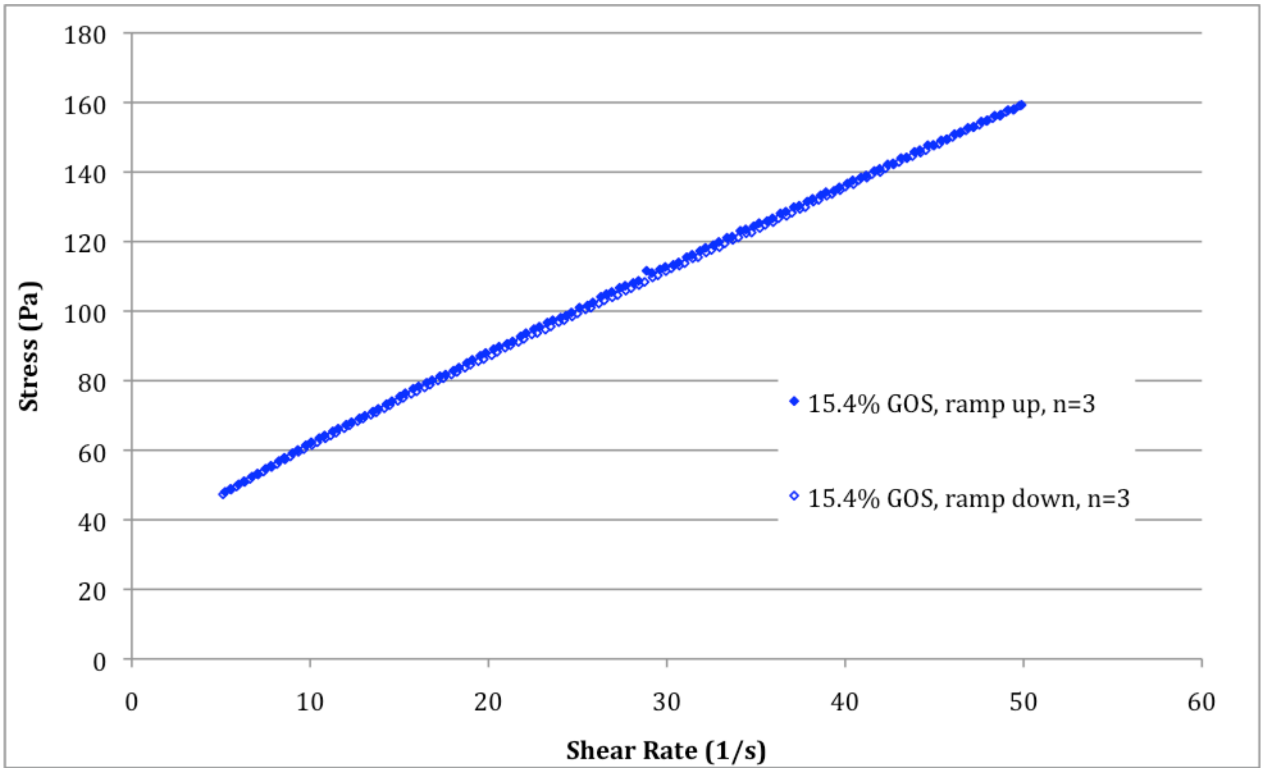

Figure 7. Stress measurements during shear ramps for 15.4% chocolate samples (Yield Stress at 5 s^{-1}).

Table 2. Rheological parameters used to define chocolate properties, α = 0.05

	Apparent Viscosity	**Yield Stress**	**Thixotropy**
Control	3.524	43.850	0.680
SD	0.025	0.286	0.231
3.75% GOS	3.611	47.200	0.303
SD	0.098	1.068	0.579
15.4% GOS	3.753	48.477	0.970
SD	0.021	0.455	0.315
30.4% GOS	3.684	47.497	0.553
SD	0.005	0.402	0.293
p-value	**<0.01**	**<0.01**	**0.061**

The viscosity flow curves and shear stress curves of Chocolate-GOS in relationship to the changing shear rate at different GOS concentration (0%, 3.75%, 15.4%, and 30.8%) are shown in Figure 5 and Figure 6, respectively. In Figure 5, the apparent viscosity values decreased with the increase in shear rate and Figure 6 shows that yield stress value increased with the increase in shear rate. Figure 5 and Figure 6 show a shear thinning behavior for all four samples, indicating that the samples are non-Newtonian fluids. As shown in Figure 5 and Figure 6, the chocolate samples with addition of GOS have slight rheological changes. It is shown that viscosity and shear stress during shear rate ramp up was increased with the increase concentration of GOS. Addition of GOS had shown to have increase in viscosity and yield stress.

When GOS was added to replace 80% of sucrose (30.4% GOS of total mass), the increase in viscosity and shear stress was less than the chocolate sample with 15.4% GOS in chocolate. This tendency is in reasonably good agreement with that found in supersaturated sucrose solution that the rheology properties could be changed when the supersaturated solution is altered (Quintas and others 2005).

Figure 7 is a representation of both ramp up and ramp down for all of the samples. All of the samples had nearly identical path for both ramp-up and ramp down. The samples are shown to return to its original states upon removal of shear. This indicates that the samples have a similar thixotropic nature. With a similar thixotropic nature, it indicates that the conching process was performed successfully.

The rheological parameters used to compare the four samples are presented in Table 2. There was a significant increase in yield stress and apparent viscosity with addition of GOS to the chocolate system. The thixotropy was not statistically different between the two samples, which indicate a successful conching process. Even though a statistical difference occurs between the yield stress and apparent viscosity this does not translate to the larger effects seen when fat and emulsifier contents are altered (Karnjanolarn and McCarthy 2006). In addition, a sensory analysis was performed and had shown that there were no statistical difference for overall liking between the control sample and 3.75% GOS sample (Suter 2010). In conclusion, yield stress and viscosity properties of Chocolate-GOS were influenced by GOS concentration, however, the rheological difference between the samples should not be considered large in relation to their parameters.

IV. Conclusion

Overall, the result of this study has shown a positive result of incorporation of GOS in the chocolate system. Chocolate can serve as an excellent delivery system for GOS. Although increases in yield stress and apparent viscosity were observed upon GOS addition, it was noted that these increases did not contribute to a noticeable quality/ textural change. Therefore, a functional chocolate containing GOS can be produced both by looking at the processing and the quality of the chocolate. Future work will consist a sensory testing when GOS inclusions at a higher level is approved to observe if chocolate with higher GOS inclusion can meet commercial standards for taste.

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